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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of	) Group Art Unit: 1645
M. Philipp	) Examiner: R. Swartz
Appln. No. 09/445,803	)
Filed: December 13, 1999	)
For: SURFACE ANTIGENS AND PROTEINS USEFUL IN COMPOSITIONS FOR THE DIAGNOSIS AND PREVENTION OF LYME DISEASE	)

Commissioner for Patents
Washington, DC 20231

DECLARATION UNDER 37 CFR § 1.132

Sir:

I, Mario T. Philipp, Ph.D., residing at 248 Shaunell Drive, Mandeville, Louisiana, USA, a citizen of the Republic of Germany, do declare and state that:

1. I am the named inventor of the subject matter claimed in the above-identified patent application

2. I understand that this Declaration is being submitted to provide additional evidence obtained after the priority date of the above-identified application, which evidence supports the disclosure of the specification. Specifically, the evidence supports

the disclosure that fragments of P7-1 SEQ ID NOS:1 and 2 are useful in the diagnostic methods for diagnosing Lyme Disease.

3. With regard to providing additional evidence, I hereby submit that fragments of the P7-1 clone have been synthesized as peptides and can be utilized for the serologic detection of Lyme Disease.

4. Exhibit A is a copy of US Patent No. 6,475,492 ('492) which was filed by me after the priority date of the present application and describes fragments of the P7-1 clone as noted in Table 1. '492 also discusses that these peptide fragments detect antibodies to the causative agent of Lyme Disease and are therefore useful for the diagnosis of the same.

Table 1

Sequence	AA Position in SEQ ID NO:2	Column of '492 Patent
MKKDDQIAAAMVLRGMAKDGQFALKD	264-289	4, lines 43-44
MKKDDQIAAAMVLRGMAKDGQFALK	264-288	7, lines 23-24
KKDDQIAAAMVLRGMAKDGQFALKD	265-289	7, lines 23-24
KKDDQIAAAMVLRGMAKDGQFALK	264-288	7, lines 23-24

5. Exhibit B is a copy of one of my publications, Liang et al., "Epitope Mapping of the Immunodominant Invariable Region of *Borrelia burgdorferi* VlsE in Three Host Species", Infection and Immunity, **68**(4):2349-2352 (April 2000) which published after the priority and filing date of the present application and describes fragments of the P7-1 clone. Liang reports the 11-mer sequence AA(I or M)(A or V)LRGMAKD, i.e., AAMVLRGMAKD, on page 2351, col. 2, paragraph 2. This

sequence is a fragment of SEQ ID NO:2 of the present application and corresponds to amino acids 272-282 of SEQ ID NO:2. Further, three 14-mer peptides were reported on page 2350, Figure 1 of Liang. Since Liang discusses that the amino acid in position 11 of fragment C_6N (also in position 5 of fragment C_6M and in position 4 of fragment C_6C) can either be -Ile- or -Met- (page 2351, paragraph 2), the sequences noted in Table 2 were reported and are fragments of SEQ ID NO:2.

Table 2

Sequence	Name	AA Position in SEQ ID NO:2	Page
MKKDDQIAAAMVLR	C_6N	264-277	2350
IAAAMVLRGMAKDG	C_6M	270-283	2350
LRGMAKDGQFALKD	C_6C	276-289	2350

Antibody to all three of these 14-mer fragments was found in sera from humans and mice with Lyme Disease (see Figs. 3A and 4, respectively, of Liang).

6. Additional data are provided which I obtained subsequent to the filing of the present application. Specifically, the following data supports the utility of the peptide fragments C_6N, C_6M, and C_6C of Liang in diagnosing Lyme Disease and provides data for an additional peptide fragment, i.e., the C_6S fragment DQIAAAMVLRGMAK, which corresponds to amino acids 268-281 of SEQ ID NO: 2. The following experiments were performed by me or under my direction.

To assess the antigenicity of the C_6N, C_6M, C_6C and C_6S fragments, thirty (30) serum samples from patients with clinically confirmed Lyme disease were tested by enzyme-linked immunosorbent assay (ELISA), using as antigens the biotinylated peptides attached to streptavidin-coated ELISA plates. All of these peptides were biotinylated at

the amino terminus.

The ELISA protocol was performed by coating Ninety-six (96) well ELISA plates (Corning Inc., Corning, NY) with 100 µL per well of 4-µg/ml streptavidin (Pierce Chemical Company, Rockford, IL) in coating buffer (0.1 M carbonate buffer, pH 9.2) and incubated at 4°C overnight. After two 3-min washes with 200 µL per well of PBST (10 mM sodium phosphate, 150 mM NaCl and 0.1% Tween-20, pH 7.4) at 200 rpm in a rotatory shaker (Orbital Shaker, Lab-Line Instruments Inc., Melrose Park, IL), 200 µL of 5 µg/ml biotinylated peptide dissolved in blocking buffer (PBST supplemented with 5% nonfat dry milk, (Carnation, Nestle Food Company, Glendale, CA)) was applied to each well. The plate was shaken at 150 rpm for 2 hours at room temperature (RT). After three washes with PBST as above, 50 µL of patient serum diluted 1:800 with blocking buffer was added to each well. The plate was incubated at 150 rpm for 1 hour at RT and then washed three times with PBST. Each well then received 100 µL of 0.1-µg/ml goat antihuman IgG (heavy and light-chain specific, Pierce), each conjugated to horseradish peroxidase and dissolved in blocking buffer. Incubation was at RT for one (1) additional hour, while shaking at 150 rpm. After four (4) washes with PBST for 3, 4, 5 and 6 minutes, respectively, 100 µL of a solution composed of the chromogen 3,3', 5,5'-tetramethylbenzidine at 0.2 mg/mL and 0.01% hydrogen peroxide in the buffer supplied by the manufacturer (Kirkegaard & Perry, Gaithersburg, MD) was added, and color was allowed to develop for 10 minutes. The enzyme reaction was stopped by addition of 100 µL of 1-M phosphoric acid. Optical density was measured at 450 nm with a microtest plate SLT-Spectra™ spectrophotometer model was obtained using the soft2000™ software (SLT Lab Instruments, Germany).

The OD of each of the 30 serum samples from Lyme disease patients was determined in duplicate with each of the peptides. In addition, each of the peptides was reacted, in duplicate, with ten (10) serum specimens obtained from ten (10) individuals who resided in an area where Lyme disease is not endemic. One ELISA plate was used

for each of the peptides. In addition to the 30 duplicate patient specimens (60 wells) and the ten (10) duplicate negative control serum specimens (20 wells) that were placed on each plate, five (5) wells were coated with the C_6 peptide and reacted with a pool of 5 human serum specimens from patients with Lyme disease. The mean OD value for the 5 repeated samples was obtained for each plate and the mean of this mean was calculated for all of the plates utilized. This mean OD value was considered the reference OD and for each plate a correcting or normalizing factor (to correct for plate variations) was calculated by dividing the mean quintuplicate OD value obtained for the serum pool of that plate by the reference OD value. This correction factor was then multiplied by the mean OD value of each of the duplicate serum samples. The cut-off OD value, above which a normalized (corrected for plate variation) OD value was considered as positive, was defined as the mean of the normalized OD of the 10 control specimens plus 3 standard deviations of that mean. The values that were listed in the tables shown below are the corrected mean OD values for each duplicate determination minus the corresponding corrected cut-off value.

The results are shown in Table 3. I confirmed that the C_6 fragments (C_6M, C_6S, C_6C, and C_6N) were antigenic in humans, as was the C_6 peptide. The mean OD value for the C_6M fragment was 0.667, and 29 of the 30 serum samples (97%) reacted with this fragment. For the C_6S fragment the mean OD value was 0.489, and 30/30 (100%) samples reacted with this fragment. For the C_6C and C_6N fragments, the mean OD values were 0.0383 and 0.0423, respectively, and 24/30 (80%) and 7/30 (23%) of the serum samples reacted with C_6C and C_6N, respectively.

Table 3					
Serum Peptide	**C_6**	**C_6M**	**C_6S**	**C_6C**	**C_6N**
1	1.282	0.314	0.229	0.019	0.000
2	1.985	0.19	0.478	0.042	0.000
3	2.200	1.666	0.862	0.001	0.000
4	2.188	0.756	0.565	0.016	0.095
5	0.988	0.015	0.004	0.025	0.000
6	2.332	1.069	0.656	0.000	0.042
7	2.256	0.789	0.758	0.016	0.781
8	2.230	0.01	0.081	0.048	0.041
9	2.424	1.409	1.132	0.123	0.087
10	2.171	1.08	0.81	0.039	0.032
11	0.582	0.009	0.004	0.000	0.000
12	2.078	1.684	0.942	0.01	0.000
13	1.985	0.000	0.063	0.105	0.000
14	1.976	1.126	0.716	0.013	0.000
15	2.339	1.314	1.052	0.008	0.000
16	2.595	1.811	1.59	0.515	0.192
17	0.517	0.264	0.135	0.000	0.000
18	2.286	0.992	0.836	0.008	0.000
19	1.550	0.662	0.428	0.000	0.000
20	1.944	0.86	0.663	0.007	0.000
21	2.377	1.808	0.957	0.000	0.000
22	1.338	0.025	0.086	0.029	0.000
23	2.178	0.015	0.062	0.004	0.000
24	1.498	0.024	0.006	0.057	0.000
25	2.153	0.07	0.032	0.016	0.000

Table 3 (continued)					
Serum Peptide	**C_6**	**C_6M**	**C_6S**	**C_6C**	**C_6N**
26	1.267	0.11	0.051	0.025	0.000
27	0.954	0.03	0.011	0.012	0.000
28	0.812	0.036	0.015	0.000	0.000
29	2.293	0.886	0.714	0.004	0.000
30	2.303	0.99	0.727	0.009	0.000
Mean OD	1.836	0.667	0.489	0.038	0.042
% positive	100%	97%	100%	80%	23%

7. In summary, these results demonstrate that a variety of fragments of the SEQ ID NO: 2 sequence of the above-identified application are capable of reacting with antibodies from subjects with Lyme Disease, and thus are useful in diagnostic methods.

8. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 12-9-02 By: _____

Mario T. Philipp, Ph.D.